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Characterization of microsatellite loci for the stingless bee *Scaura latitarsis* (Hymenoptera, Apidae, Meliponini)

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Abstract

Seven microsatellite loci were isolated and characterized from a microsatellite enriched genomic library for the stingless bee *Scaura latitarsis*. Primers were tested in 12 individuals. The number of alleles per locus ranged from 3 to 6 (mean = 4.29) and observed heterozygosity ranged from 0.00 to 0.75 (mean = 0.40). Cross-species tests showed successful amplification for *Scaura atlantica*, *Scaura longula*, *Scaura tenuis*, *Schwarzula timida*, *Schwarziana quadripunctata*, *Plebeia droryana*, and *Plebeia remota*.

Keywords

Microsatellites, *Scaura latitarsis*, genetic variability, Meliponini

Introduction

Scaura latitarsis is small (4.0 mm) and black stingless bee which nidifies in arboreal termite nests of *Nasutitermes* (Michener 2007). The species is widely distributed in tropical South America, with records in the following countries: Colombia, Venezuela,

Guyana, Suriname, Peru, Bolivia, and Brazil (Camargo and Pedro 2012). The large geographic distribution and the well reported female philopatric behavior in stingless bees (Nogueira-Neto 1954, Brito and Arias 2010, Francisco and Arias 2010) suggest that *S. latitarsis* populations should be highly differentiated. In general, population studies apply combined molecular markers of nuclear and cytoplasm origins. Mitochondrial polymorphism is easily assessed due to universal primers already described (Simon et al. 1994). However, the same is not true for nuclear markers. Microsatellites have been the nuclear marker most used in population genetic studies. As they present Mendelian and biparental inheritance, it is possible to evaluate the parental contribution, especially from males when mitochondrial data are also available (e.g. Estoup et al. 1996, Franck et al. 2001, Quezada-Euán et al. 2011). For future population studies, our first goal was to construct a microsatellite-enriched genomic library to isolate and characterize microsatellite loci for *S. latitarsis*.

Total DNA of 12 workers was extracted using a phenol/chloroform protocol. The microsatellite-enriched genomic library was constructed according to Billotte et al. (1999) with some modifications (Brito et al. 2009). From 96 selected colonies of transformed *Escherichia coli* DH5α lineage, 48 were sequenced and 25 contained microsatellite sequences. Fifteen of these were chosen for primers design based on sequence quality. The software Primer3 was used (Rozen and Skaletsky 2000) to indicate the best primer sequences. Primers were tested on 12 workers of *S. latitarsis* from seven different locations in Brazil: Ribeirão Preto, SP (n = 2), Cajuru, SP (n = 2), Pedregulho, SP (n = 2), Ribeirão Cascalheira, MT (n = 2), Nova Xavantina, MT (n = 1), Manaus, AM (n = 2), and Londrina, PR (n = 1). Amplification reactions were conducted in a volume of 20 μL containing 2 μL of the PCR reaction buffer (Invitrogen), 1 μL of dNTP 2 mM each, 0.6 μL of $MgCl_2$ 50 mM, 0.4 μL of each primer 20 μM, 15 ng of template DNA and 0.3 μL of *Taq* DNA polymerase 5U/μL (Invitrogen). PCR conditions were the following: 93 °C for 4 min, 35 cycles of denaturation at 93 °C for 40 s, annealing at 60 °C for 50 s and elongation at 72 °C for 40 s. A final extension step of 72 °C for 5 min was performed. PCR products were separated on 5.6% polyacrylamide gels and stained with silver nitrate. Allelic richness (k), observed (H_O) and expected (H_E) heterozygosity were calculated in FOFPOP 2.0 (Francisco 2009). GENEPOP 4.1.4 (Rousset 2008) was used for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium tests. Bonferroni corrections were used for multiple comparisons.

Seven out of 15 primer pairs generated fragments of expected size, and they were polymorphic (Table 1). The number of alleles ranged from 3 to 6, with an average of 4.29. Values of H_O ranged from 0.000 to 0.750 (mean = 0.405) and H_E from 0.531 to 0.736 (mean = 0.664). After Bonferroni correction, only the loci SLAT 16 and SLAT 40 showed significant deviation from Hardy-Weinberg-expectations. Linkage disequilibrium was detected after Bonferroni correction between SLAT 16/SLAT 24, SLAT 24/SLAT 43, and SLAT 40/SLAT 43. Deviations from HWE and linkage disequilibrium are expected when individuals are sampled from genetically differentiated populations (Brito et al. 2009).

Using the same conditions aforementioned we tested the primers for the polymorphic loci in seven stingless bee species: *Scaura atlantica*, *Scaura longula*, *Scaura tenuis*, *Schwarzula timida*, *Schwarziana quadripunctata*, *Plebeia droryana*, and *Plebeia remota*. Cross-species amplification was 100% efficient for SLAT 10 and SLAT 44 (Table 2). However those loci were the only ones amplified in *S. longula* and *S. tenuis*. At least five out of seven primer pairs were successfully amplified in species from three other genera.

The microsatellite loci described here will be very helpful to gather molecular data of *S. latitarsis* to understand the population structure of this widely distributed species. If queen philopatry is confirmed for this species, these microsatellite loci will allow an evaluation of male's contributions as gene flow mediators.

Table 1. Summary of general information for the microsatellite loci isolated from *Scaura latitarsis*. *k*: number of alleles; ASR: allele size range (bp); H_O: observed heterozygosity; H_E: expected heterozygosity.

Locus	Repeat motif	Genbank accession number	Primer sequences (5'-3')	k	ASR	H_O	H_E
SLAT 10	$(AG)_{12}TA(AG)_3$	JQ001766	F: TGAATTAACAGGCCGGAATC R: TTCGTTGCCTCGCTCTTTAT	5	183–197	0.417	0.694
SLAT 16	$(AG)_{21}$	JQ001767	F: AAACGAAGGACAGACGTTGG R: AATATGTGGACCGCGTGTTA	5	145–179	0.000	0.736
SLAT 18	$(AG)_{24}$	JQ001768	F: GGATCGTCGAACGGAATATC R: TCGTCAACATTCCTCACTGG	3	232–240	0.250	0.531
SLAT 24	$(GAA)_{16}$	JQ001769	F: CGAACCAGTGTCTGAATCGT R: GCCTCGAACTCTGGCGTAT	6	184–202	0.500	0.715
SLAT 40	$(GA)_3AA(GA)_{18}$	JQ001771	F: GTTCCACACCGGAAGACC R: GTGAACGAGCCTCTGCAATC	4	200–214	0.333	0.653
SLAT 43	$(AG)_{24}$	JQ001772	F: GCGGAATTAACAACGGACAT R: GCATACCGGCGAATATAACC	4	119–135	0.583	0.719
SLAT 44	$(GA)_{19}(GAA)_9$	JQ001773	F: GGCATAGGTTACGATTTGGAG R: GTGGCAGTCAACGTGTTAGG	3	222–230	0.750	0.601

Table 2. Cross-species amplification of 7 microsatellite loci from *Scaura latitarsis* in *S. atlantica*, *S. longula*, *S. tenuis*, *Schwarzula timida*, *Schwarziana quadripunctata*, *Plebeia droryana*, and *Plebeia remota*. (+): successful amplification; (-): no product or multiple bands.

Locus	*Scaura atlantica*	*Scaura longula*	*Scaura tenuis*	*Schwarzula timida*	*Schwarziana quadripunctata*	*Plebeia droryana*	*Plebeia remota*
SLAT 10	+	+	+	+	+	+	+
SLAT 16	+	-	-	-	-	-	-
SLAT 18	-	-	-	+	+	+	+
SLAT 24	+	-	-	+	+	+	+
SLAT 40	-	-	-	+	+	+	+
SLAT 43	+	-	-	-	-	+	-
SLAT 44	+	+	+	+	+	+	+

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